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International application number: PCT/US05/011046

International filing date: 01 April 2005 (01.04.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/558,889
Filing date: 02 April 2004 (02.04.2004)

Date of receipt at the International Bureau: 06 May 2005 (06.05.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

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April 26, 2005

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APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE.

APPLICATION NUMBER: 60/558,889

FILING DATE: *April 02, 2004*

RELATED PCT APPLICATION NUMBER: PCT/US05/11046

Certified by



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040204
18334 U.S. PTO

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PTO/SB/16 (08-03)

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 367470327 US

60/558889
18334 U.S. PTO
040204

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Mark J.	Cantwell	San Diego, CA

Additional inventors are being named on the 1 separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

METHODS OF USING 5,10-METHYLENE HYDROFOLATE TO TREAT CANCER

Direct all correspondence to: CORRESPONDENCE ADDRESS

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OR

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ENCLOSED APPLICATION PARTS (check all that apply)

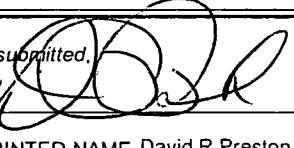
<input checked="" type="checkbox"/> Specification Number of Pages	19	<input type="checkbox"/> CD(s), Number	_____
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets	19	<input type="checkbox"/> Other (specify)	_____
<input type="checkbox"/> Application Date Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.	FILING FEE Amount (\$)
<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees.	80.00
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Respectfully submitted,
SIGNATURE 
TYPED or PRINTED NAME David R Preston

[Page 1 of 2]

Date April 2, 2004

REGISTRATION NO. 38,710
(if appropriate)
Docket Number: ADX-00101.P.1

TELEPHONE 858-724-0375 x102

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Additional Page

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Docket Number ADX-00101.P.1

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Joan M.	Robbins	San Diego CA

[Page 2 of 2]

Number 1 of 1

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FEE TRANSMITTAL

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Effective 01/01/2003. Patent fees are subject to annual revision.

Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT **(\$)** 80.00

Complete if Known

Application Number	To be determined
Filing Date	Herewith
First Named Inventor	CANTWELL
Examiner Name	To be determined
Art Unit	To be determined
Attorney Docket No.	ADX-00101.P.1

METHOD OF PAYMENT (check all that apply)

Check Credit card Money Order Other None

 Deposit Account:

Deposit Account Number 501321
Deposit Account Name David R. Preston

The Commissioner is authorized to: (check all that apply)
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FEE CALCULATION**1. BASIC FILING FEE**

Large Entity	Small Entity	Fee Code (\$)	Fee Code (\$)	Fee Description	Fee Paid
1001 750	2001 375			Utility filing fee	
1002 330	2002 165			Design filing fee	
1003 520	2003 260			Plant filing fee	
1004 750	2004 375			Reissue filing fee	
1005 160	2005 80			Provisional filing fee	80.00
SUBTOTAL (1)		(\$) 80.00			

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims	Independent Claims	Multiple Dependent	Extra Claims	Fee from below	Fee Paid
			-20** =	X	
			-3** =	X	

Large Entity	Small Entity	Fee Code (\$)	Fee Code (\$)	Fee Description	Fee Paid
1202 18	2202 9			Claims in excess of 20	
1201 84	2201 42			Independent claims in excess of 3	
1203 280	2203 140			Multiple dependent claim, if not paid	
1204 84	2204 42			** Reissue independent claims over original patent	
1205 18	2205 9			** Reissue claims in excess of 20 and over original patent	
SUBTOTAL (2)		(\$) 0.00			

**or number previously paid, if greater. For Reissues, see above

3. ADDITIONAL FEES

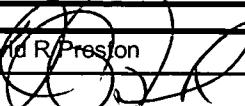
Large Entity	Small Entity	Fee Code (\$)	Fee Code (\$)	Fee Description	Fee Paid
1051 130	2051 65			Surcharge - late filing fee or oath	
1052 50	2052 25			Surcharge - late provisional filing fee or cover sheet	
1053 130	1053 130			Non-English specification	
1812 2,520	1812 2,520			For filing a request for ex parte reexamination	
1804 920*	1804 920*			Requesting publication of SIR prior to Examiner action	
1805 1,840*	1805 1,840*			Requesting publication of SIR after Examiner action	
1251 110	2251 55			Extension for reply within first month	
1252 410	2252 205			Extension for reply within second month	
1253 930	2253 465			Extension for reply within third month	
1254 1,450	2254 725			Extension for reply within fourth month	
1255 1,970	2255 985			Extension for reply within fifth month	
1401 320	2401 160			Notice of Appeal	
1402 320	2402 160			Filing a brief in support of an appeal	
1403 280	2403 140			Request for oral hearing	
1451 1,510	1451 1,510			Petition to institute a public use proceeding	
1452 110	2452 55			Petition to revive - unavoidable	
1453 1,300	2453 650			Petition to revive - unintentional	
1501 1,300	2501 650			Utility issue fee (or reissue)	
1502 470	2502 235			Design issue fee	
1503 630	2503 315			Plant issue fee	
1460 130	1460 130			Petitions to the Commissioner	
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1806 180	1806 180			Submission of Information Disclosure Stmt	
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1809 750	2809 375			Filing a submission after final rejection (37 CFR 1.129(a))	
1810 750	2810 375			For each additional invention to be examined (37 CFR 1.129(b))	
1801 750	2801 375			Request for Continued Examination (RCE)	
1802 900	1802 900			Request for expedited examination of a design application	

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SUBTOTAL (3) **(\$)** 0.00**SUBMITTED BY**

(Complete if applicable)

Name (Print/Type)	David R. Preston	Registration No. (Attorney/Agent)	38,710	Telephone 858-724-0375
Signature				
Date	12/22/2004			

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Mail Stop Provisional Application
"Express Mail" Mailing Label Number: EV 367470327 US

Date of Deposit: April 2, 2004

Commissioner for Patents
Alexandria, VA 22313-1450

Re: Provisional Patent Application
Entitled: METHODS OF USING 5,10-METHYLENE HYDROFOLATE
TO TREAT CANCER
Appl. No.: To be determined
Filed: Herewith
Inventor: CANTWELL, Mark; ROBBINS, Joan
Our Ref.: ADX-00101.P.1

Sir:

The following documents are forwarded herewith for appropriate action by the United States Patent and Trademark Office:

1. Provisional Application for Patent Cover Sheet (in duplicate);
2. Fee transmittal (in duplicate);
3. Complete U.S. Provisional Patent Application entitled:

**METHODS OF USING 5,10-METHYLENE HYDROFOLATE TO
TREAT CANCER**

and naming as inventors

CANTWELL, Mark; ROBBINS, Joan

the provisional application comprising:

1. Total pages of application: [39];
2. Pages of specification: [19];
3. Sheets of Figures: [19];
4. Pages of Title Page: [1];

4. One Return Post Card; and

5. Our Check for \$80.00 to cover the Application Fee.

It is respectfully requested that the attached postcard be stamped with the filing date and unofficial application number and returned as soon as possible.

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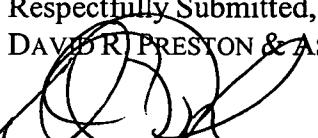
The following attorney is the attorney of record for prosecuting this application and transacting all business in the USPTO connected therewith:

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Respectfully Submitted,
DAVID R. PRESTON & ASSOCIATES, A.P.C.


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Registration No. 38,710

PROVISIONAL
PATENT APPLICATION
on
METHODS OF USING 5,10-METHYLENE HYDROFOLATE TO TREAT
CANCER
by

Mark J. Cantwell and Joan M. Robbins

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ADX-00101.P.1

METHODS OF USING 5,10-METHYLENE TETRAHYDROFOLATE TO TREAT CANCER

Cancer is a major public health concern. Colorectal cancer alone cases approximately 50,000 deaths per year in the United States. Nearly half of the 5 approximately 130,000 cases of colorectal cancer that are diagnosed every year present with or develop into metastatic disease, for which chemotherapy is the only treatment. New effective drug-based therapies for treatment are urgently sought not only for colorectal cancers, but for other cancers such as but not limited to breast cancer, pancreatic cancer, stomach cancers, hepatic cancer, bladder cancer, cervical cancer, head 10 and neck cancer, lung cancer, ovarian cancer, and prostate cancer. The present invention provides new drug-based methods of cancer treatment, including methods that can provide reduced toxicity to the patient and greater efficacy than current modalities.

The anticancer drug 5-fluorouracil (5-FU) is an inhibitor of thymidylate synthase (TS), an enzyme required for nucleic acid biosynthesis. 5-FU used to treat cancers such 15 as colorectal and breast cancer, is commonly used in conjunction with folinic acid (leucovorin), which is converted intracellularly into reduced folate, a cofactor for TS. Toxicities associated with 5-fluorouracil include stomatitis, mucositis, gastrointestinal symptoms, and hematological toxicity, particularly neutropenia, thrombocytopenia, and leucopenia.

20 There is a need to develop improved anti-cancer drug regimens that increase survivorship with reduced toxicity. Clinical trials have demonstrated that administration of 5,10-methylene tetrahydrofolate, a form of reduced folate used as a cofactor by TS, along with 5-FU, increases the length of remissions in patients with breast and gastrointestinal cancer when compared with the use of folinic acid (leucovorin) combined 25 with 5-FU.

Detailed Description of the Invention

The present invention is based on the surprising result that 5,10-methylene tetrahydrofolate (5,10-CH₂-THFA), while increasing the efficacy of 5-fluorouracil (5-FU) in reducing the rate of tumor growth and increasing survivorship, also reduces the 5 toxicity to the patient of 5-FU. As disclosed herein, treatment with 5,10-CH₂-THFA and 5-FU reduces tumor growth rate and increases survivorship of tumor-bearing animals with respect to treatment with either 5-FU alone, or 5-FU in combination with folinic acid (FA; leucovorin), while demonstrating less toxicity than either treatment.

The present invention is further based on the finding that treatment of tumor-bearing animals with 5,10-CH₂-THFA and 5-FU and additional anticancer drugs can also improve outcomes with respect to single modality treatments or alternative combination treatments that include the use of 5-FU with folinic acid (leucovorin).

The present invention provides:

- 15 1. Methods for decreasing the toxicity to a patient of a cancer drug treatment regimen that includes administration of 5-fluorouracil (5-FU) to a cancer patient by co-administering 5,10-methylene tetrahydrofolate (5,10-CH₂-THFA). The methods include treatments in which the toxicity of treatment with 5-FU is reduced by administering 5,10-CH₂-THFA instead of folinic acid as a source of TS cofactor.
- 20 2. Methods of treating cancer patients with combination chemotherapy involving 5-fluorouracil (5-FU), 5,10-methylene tetrahydrofolate (5,10-CH₂-THFA), and one or more additional anti-cancer drugs. Treating cancer patients with 5,10-CH₂-THFA, 5-FU, and one or more additional anti-cancer drugs can reduce the rate of tumor growth or increase 25 the survivorship of cancer patients when compared with treating patients with the one or more additional anti-cancer drugs in the absence of 5,10-CH₂-THFA and 5-FU, or when compared with treating patients with 5-FU and the one or more additional anti-cancer drugs in the absence of 5,10-CH₂-THFA, or when compared with treating patients with 5-FU and folinic acid and the one or more additional anti-cancer drugs.
- 30 Treating cancer patients with 5,10-CH₂-THFA, 5-FU, and one or more additional anti-cancer drugs can reduce the toxicity of treatment when compared with treating

patients with 5-FU and the one or more additional anti-cancer drugs in the absence of 5,10-CH₂-THFA, or when compared with treating patients with 5-FU and folinic acid and the one or more additional anti-cancer drugs.

5 **I. METHODS FOR DECREASING THE TOXICITY TO A PATIENT OF A CANCER DRUG
TREATMENT REGIMEN THAT INCLUDES ADMINISTRATION OF 5-FLUOROURACIL (5-FU)
BY CO-ADMINISTERING 5,10-METHYLENE TETRAHYDROFOLATE (5,10-CH₂-THFA)**

One aspect of the present invention is methods for decreasing the toxicity of a cancer drug treatment that includes administration of 5-fluorouracil (5-FU). The method 10 comprises administering 5,10-methylene tetrahydrofolate (5,10-CH₂-THFA) to the patient before, after, or concurrent with the administration of 5-FU to reduce the toxicity of 5-FU. In preferred embodiments of this aspect of the present invention, 5-FU and 5,10-CH₂-THFA are administered to the patient in the absence of folinic acid (FA; leucovorin). In some preferred embodiments of this aspect of the present invention, 5,10- 15 CH₂-THFA is administered to a patient receiving 5-FU to reduce hematological toxicity of 5-FU. In some preferred embodiments of this aspect of the present invention, 5,10-CH₂-THFA is administered to a patient receiving 5-FU and a TS cofactor or cofactor precursor, where 5,10-CH₂-THFA is administered instead of folinic acid (FA, leucovorin), to prevent the hematological toxicity associated with treatment with 5-FU 20 and a TS cofactor (or cofactor precursor).

The invention is based on the surprising result that 5,10-methylene tetrahydrofolate, while increasing the efficacy of 5-FU in reducing the rate of tumor growth and increasing survivorship, also reduces the toxicity of 5-FU towards nontumor cells. As disclosed in Examples 1 and 2, treatment with 5,10-CH₂-THFA and 5-FU 25 reduces tumor growth rate and increases survivorship of tumor-bearing animals with respect to treatment with either 5-FU alone, or 5-FU in combination with folinic acid (leucovorin), while demonstrating less toxicity to the animal than either treatment.

As used herein, “reduce the toxicity” refers to reducing toxic systemic effects on the patient, or toxic effects on the noncancerous cells of the patient. Toxicity can include, 30 as nonlimiting examples, increased lacrimation; mucositis; esophagopharyngitis; neurological toxicity, such as parasthesias, insomnia, and dizziness; gastrointestinal

toxicity, such as nausea, vomiting, and diarrhea; cardiac toxicity; dermatological toxicity, including alopecia, sweating, and rashes; and hematological toxicity, such as, but not limited to, neutropenia, thrombocytopenia, lymphopenia, and leucopenia.

In preferred embodiments of this aspect of the present invention, 5,10-CH₂-THFA 5 is administered along with 5-FU to reduce the degree of hematological toxicity associated with 5-FU treatment. For example, administering 5,10-CH₂-THFA along with 5-FU can reduce neutropenia, thrombocytopenia, lymphopenia, or leucopenia associated with chemotherapy regimens that include 5-FU, including but not limited to chemotherapy regimens that include 5-FU and folinic acid (leucovorin).

10 A cancer patient can be a patient with any type of cancer. In some preferred embodiments of the present invention in which 5,10-CH₂-THFA is administered to a cancer patient receiving 5-FU, the patient has a tumor type that is currently treated with 5-FU, such as, for example, colorectal carcinoma, pancreatic, breast, or stomach cancer.

Those skilled in the art of cancer treatment and chemotherapy would be able to 15 determine optimal dosages and regimens for 5,10-CH₂-THFA and 5-FU. Some preferred treatments of cancer patients with 5-FU and 5,10-CH₂-THFA are regimens using from 10 milligrams to 1 gram of 5,10-CH₂-THFA per m², preferably from 25 milligrams to 500 milligrams of 5,10-CH₂-THFA per m², and more preferably from about 50 milligrams to about 250 milligrams of 5,10-CH₂-THFA per m². For example, a preferred dose of 5,10- 20 CH₂-THFA can be from about 100 to about 200 milligrams per m². Dosage of 5-FU can be from about to about 25 milligrams to about 5 grams per m², and is preferably from about 50 milligrams to 2.5 grams per m², and more preferably from about 100 milligrams to about 1 gram per m². For example, a preferred dose of 5-FU can be from about 250 to about 700 milligrams per m².

25 The drugs can be administered intravenously or by any other feasible means, according to regimens that can be determined by qualified clinicians. For example, bolus injection of each drug can be given once weekly for a number of weeks. Preferably, 5,10-CH₂-THFA is administered prior to 5-FU. For example, the patient can receive the 5,10-CH₂-THFA dose from about 10 minutes to about four hours prior to receiving the 5-FU 30 dose. We also propose 5,10-CH₂-THFA use with new formulations of 5-FU, specifically oral forms of 5-FU (e.g. Xeloda, capecitabine).

II. METHODS OF TREATING CANCER PATIENTS WITH COMBINATION CHEMOTHERAPY INVOLVING 5-FLUOROURACIL (5-FU), 5,10-METHYLENE TETRAHYDROFOLATE (5,10-CH₂-THFA), AND ONE OR MORE ADDITIONAL ANTI-CANCER DRUGS.

5 One aspect of the present invention is methods for treating cancer patients with combination chemotherapy that includes administration of 5-fluorouracil (5-FU), 5,10-CH₂-THFA, and one or more additional anti-cancer drugs. The method comprises administering 5-FU, 5,10-CH₂-THFA, and one or more additional drugs to a cancer patient in the absence of folinic acid (leucovorin). As used herein, an “additional” anti-
10 cancer drug is an anti-cancer drug that is not 5,10-methylene tetrahydrofolate (5,10-CH₂-THFA), 5-fluorouracil (5-FU), or folinic acid (FA; leucovorin).

An anti-cancer drug can be any drug used to treat cancer, including small molecules, large molecules, peptides, nucleic acids and nucleic acid analogues (such as, but not limited to antisense molecules, ribozymes, and siRNAs), and antibodies or
15 antibody fragments. As nonlimiting examples, anticancer drugs used in combination therapy with 5-FU and 5,10-CH₂-THFA can be topoisomerase inhibitors (e.g., irinotecan), antimetabolite drugs (e.g., methotrexate, gemcitabine), alkylating agents (e.g., cyclophosphamide), nucleic acid biosynthesis inhibitors (e.g., mitomycin, doxorubicin, cisplatin, oxaliplatin), microtubule disrupting drugs (e.g., paclitaxel, pa-
20 vincristine), hormone blocking drugs (e.g., tamoxifen), inhibitors of kinases, including but not limited to receptor and nonreceptor tyrosine kinases (e.g., Iressa, Tarceva, SU5416, PTK787, Gleevec), proteosome inhibitors (e.g., bortezomib), immune modulators (e.g., levamisole), cytokines (e.g., interleukins, tumor necrosis factors) and drugs that inhibit the activity of cytokines, hormones, or receptors for cytokines or
25 hormones (e.g., bevacizumab, avastin). An anti-cancer drug can also be a drug under investigation for potential anti-cancer activity, such as those listed in **Table 1**. Anti-cancer drugs include monoclonal antibodies, such as but not limited to monoclonal antibodies that bind cytokines, hormones, or hormone receptors (e.g., antibodies that block activation of EGF or VEGF growth factors, such as Avastin, erbitux, herceptin),
30 etc.

A cancer patient can be a patient with any type of cancer. In some preferred embodiments of the present invention in which 5,10-CH₂-THFA is administered to a cancer patient receiving 5-FU, the patient has a tumor type that is currently treated with 5-FU, such as, for example, colorectal carcinoma, pancreatic, breast, or stomach cancer.

5 The inventors also contemplate that combination therapies that use 5,10-CH₂-THFA, 5-FU, and one or more additional anti-cancer drugs have potential for treating cancers other than those currently commonly treated with 5-FU.

In some embodiments of this aspect of the present invention, treating a cancer patient with 5,10-CH₂-THFA, 5-FU, and one or more additional anti-cancer drugs can 10 reduce the rate of tumor growth in a cancer patient when compared with treating the patient with the one or more additional anti-cancer drugs in the absence of 5,10-CH₂-THFA and 5-FU, or when compared with treating a patient with 5-FU and the one or more additional anti-cancer drugs in the absence of 5,10-CH₂-THFA.

In some embodiments of this aspect of the present invention, treating cancer 15 patients with 5,10-CH₂-THFA, 5-FU, and one or more additional anti-cancer drugs can increase the survivorship of cancer patients when compared with treating cancer patients with the one or more additional anti-cancer drugs in the absence of 5,10-CH₂-THFA and 5-FU, or when compared with treating cancer patients with 5-FU and the one or more additional anti-cancer drugs in the absence of 5,10-CH₂-THFA.

20 In some embodiments of this aspect of the present invention, addition of 5,10-CH₂-THFA to a treatment regimen that includes 5-FU and an additional anti-cancer drug can reduce the toxicity to the patient of treatment with 5-FU and one or more additional anti-cancer drugs. Thus, the present invention includes a method of reducing the toxicity to the patient of a drug regimen for cancer treatment that includes 5-FU and one or more 25 additional anti-cancer drugs, comprising adding to the drug regimen 5,10-CH₂-THFA. In some embodiments, the reduced toxicity of 5-FU when combined with 5,10-CH₂-THFA can permit drug regimens in which 5,10-CH₂-THFA and 5-FU are used in combination with the one or more additional anti-cancer drugs that would be prohibitively toxic in the absence of CH₂-THFA.

30 In embodiments in which addition of 5,10-CH₂-THFA to a treatment regimen that includes 5-FU and an additional anti-cancer drug can reduce the toxicity to a patient of

treatment with 5-FU and the additional anti-cancer drug, the inventors contemplate that dosage of at least one of the one or more additional anti-cancer drugs can be administered at an increased dosage relative to the dosage typically used for the one or more additional anti-cancer drugs. Thus, the invention includes a method of increasing the dosage of at least one additional anti-cancer drug used in a drug regimen for treating cancer that includes 5-FU, comprising adding to the drug regimen 5,10-CH₂-THFA.

For example, because of the anti-tumor activity and decreased systemic toxicity of 5,10-CH₂-THFA compared to folinic acid (leucovorin), and because of the similar chemical and metabolic pathways of folinic acid and 5,10-CH₂-THFA, we hypothesize

5 5,10-CH₂-THFA can substitute for leucovorin in a range of current chemotherapy regimens. Current drugs commonly used in combination with 5-FU plus leucovorin are Irinotecan (CPT-11) and Oxaliplatin. The present invention includes treatments that substitute 5,10-CH₂-THFA for leucovorin in these regimens. Substitution of 5,10-CH₂-THFA for leucovorin can provide equivalent or enhanced therapeutic effects with 10 reduced toxicity. As nonlimiting examples, current drug combination regimens that 5,10-CH₂-THFA can substitute for leucovorin include:

- AIO regimen (folic acid, 5-FU, Irinotecan):

20

- Irinotecan (100 mg/m²) as a 2-hour infusion day 1; leucovorin (500 mg/m²) as a 2-hour infusion day 1; followed by 5-FU (2,000 mg/m²) intravenous (IV) bolus via ambulatory pump over 24 hours weekly x 4 every 52 weeks.

- Douillard regimen (folic acid, 5-FU, Irinotecan):

25

- Irinotecan (180 mg/m²) as a 2-hour infusion day 1; leucovorin (200 mg/m²) as a 2-hour infusion days 1 and 2; followed by a loading dose of 5-FU (400 mg/m²) IV bolus, then 5-FU (600 mg/m²) via ambulatory pump over 22 hours days 1 and 2 every 2 weeks.

30

- FOLFOX4 regimen (oxaliplatin, leucovorin, 5-FU):

- Oxaliplatin (85 mg/m²) as a 2-hour infusion day 1; leucovorin (200 mg/m²) as a 2-hour infusion days 1 and 2; followed by a loading dose of 5-FU (400 mg/m²) IV bolus, then 5-FU (600 mg/m²) via ambulatory pump over 22 hours days 1 and 2 every 2 weeks.

- FOLFOX6 regimen (oxaliplatin, leucovorin, 5-FU):
 - Oxaliplatin (85-100 mg/m²) as a 2-hour infusion day 1; leucovorin (400 mg/m²) as a 2-hour infusion day 1; followed by a loading dose of 5-FU (400 mg/m²) IV bolus on day 1, then 5-FU (2,400-3,000 mg/m²) via ambulatory pump over 46 hours every 2 weeks.
- FOLFIRI regimen (folic acid, 5-FU, Irinotecan):
 - Irinotecan (180 mg/m²) as a 2-hour infusion day 1; leucovorin (400 mg/m²) as a 2-hour infusion day 1; followed by a loading dose of 5-FU (400 mg/m²) IV bolus on day 1, then 5-FU (2,400-3,000 mg/m²) via ambulatory pump over 46 hours every 2 weeks.
- IFL (or Saltz) regimen (Irinotecan, 5-FU, leucovorin):
 - Irinotecan (125 mg/m²), 5-FU (500 mg/m²) IV bolus, and leucovorin (20 mg/m²) IV bolus weekly for 4 out of 6 weeks.

The forgoing examples are not intended to be limiting in any way. For example, dosages and regimens can be altered or optimized to minimize toxicity to the patient or improve efficacy. In addition, many anti-cancer drugs that are not described herein can be combined with 5,10-CH₂-THFA and 5-FU. We also propose 5,10-CH₂-THFA use in combination therapies with next-generation forms of 5-FU, specifically oral forms of 5-FU (e.g. Xeloda, capecitabine).

Other uses of 5,10-CH₂-THFA are in combination therapy with new classes of biologic anti-tumor reagents, such as monoclonal antibodies with anti-tumor activity. Examples of antibodies that might be combined with 5,10-CH₂-THFA (preferably with 5-FU) include anti-VEGF antibody (e.g. Avastin, Bevacizumab) and anti-EGF receptor (e.g. Erbitux, cetuximab, herceptin). As shown in the Examples, combination 5-FU/5,10-CH₂-THFA /Avastin treatment of colorectal carcinoma in nude mice inhibits tumor growth more than the other drug combinations.

Because of the lower toxicity profile of 5,10-CH₂-THFA disclosed herein, the present invention also includes 5,10-CH₂-THFA use in combination with drugs that typically are considered too toxic for widespread use. For example, 5-FU/5,10-CH₂-THFA /Cisplatin therapy is a hypothetical combination. Cisplatin, a platinum-based chemotherapy agent is highly toxic. In addition, the lower toxicity profile of 5,10-CH₂-THFA might

allow use of either increased concentrations of drugs (e.g. 5-FU) or prolonged dosing periods. In turn this might improve drug efficacy.

The present invention also includes the use of 5,10-CH₂-THFA in place of folinic acid (leucovorin) in therapies that do not use 5-FU. For example, based on the lower 5 toxicity profile and increased activity of 5,10-CH₂-THFA (CoFactor) compared to folinic acid (leucovorin), 5,10-CH₂-THFA can be used for methotrexate rescue therapy. This mode of therapy currently uses leucovorin.

10 **EXAMPLE 1: NUDE MOUSE STUDY ON COLORECTAL TUMOR HT-29 TREATMENT WITH 5-FU, 5,10-CH₂-THFA, FA, ANTI-VEGF, AND OXALIPLATIN.**

Materials and Methods

Mice

15 Nude (nu/nu) mice were obtained from Charles River Laboratories. Mice were 6-8 weeks old at the start of all studies. Mice were maintained in isolated, hepa-filter ventilated cages with 4 mice per cage at LAB International's vivarium (San Diego, CA).

Cell Lines

20 The human colon carcinoma HT-29 was obtained from American Tissue Culture Collection (ATCC). Cell lines were maintained in DMEM containing 10% fetal bovine serum (FBS), 2mM l-glutamine, 100 units/ml penicillin, and 100 micrograms/ml streptomycin (DMEM-10) in a 37°C, 5% CO₂ humidified incubator. Cell lines were passaged every 2-3 days prior to *in vivo* experiments.

25

Drugs

5-Fluorouracil (5-FU) was obtained from Calbiochem. Folinic acid (leucovorin) and oxaliplatin were obtained from Sigma-Aldrich. CoFactor (5,10 methylenetetrahydrofolate) was manufactured by Eprova AG. A monoclonal antibody to 30 vascular endothelial growth factor (anti-VEGF) was either obtained from R&D Systems (clone 26503 recognizing the human VEGF isoform 165) or Genentech (Avastin).

HT-29 Colorectal Carcinoma Nude Mouse Study #1

HT-29 cells were prepared for injection as follows. Confluent tissue culture flasks of HT-29 cells were washed once with PBS followed by cell detachment with trypsin. Detached cells were then washed once in DMEM-10 followed by one wash with 5 PBS. Finally, cells were resuspended at 2×10^7 cells/ml in PBS. Nude mice (nu/nu) were inoculated subcutaneously with 100 microliters (2×10^6 cells) of HT-29 cells using a 28 gauge insulin needle/syringe. When tumors reached 100 to 300 mm³ in volume, mice were treated with various combinations of 5-FU, CoFactor, leucovorin, oxaliplatin, and anti-VEGF (R&D Systems antibody) administered by intraperitoneal injection. All drugs 10 were dosed daily (0.6 mg/mouse/drug) for five consecutive days with the exception of anti-VEGF and oxaliplatin. Anti-VEGF was dosed once (100 microgram/mouse) on day 5. Oxaliplatin was dosed once on day 1 (0.3mg/mouse). In addition, CoFactor or leucovorin were injected 20 minutes prior to 5-FU injection. Tumor sizes were measured 15 every 2 to 3 days using calipers. Tumor volume was calculated using the following formula: tumor volume = (length x width²)/2. Mice were euthanized by CO₂ followed by cervical dislocation either when a tumor reached >2cm in diameter or upon tumor ulceration.

Data Analysis

20 Statistical analysis of tumor and blood data was performed using GraphPad Prism scientific software. Bonferonni's T test was used to compare tumor sizes between multiple groups. The Logrank test was used to determine statistical differences between group survival curves. In some cases, in which only two groups were compared, Student's T test was used to determine the significance between group measurements.

25

Results

Nude mice were treated with the drug combinations described in **Table 2**. In this 30 study, we wanted to examine if combining 5-FU/CoFactor treatment with the oxaliplatin or anti-VEGF antibody (obtained from R&D Systems) could inhibit colorectal tumor growth more than other drug combinations. Drug concentrations and treatment days are described in the materials and methods section. Following treatment, tumor sizes were

measured every 2-3 days and tumor volumes calculated. Tumor volumes were then plotted versus time from treatment initiation (**Figures 1 and 2**). To simplify the graphs, we divided analysis into graphs containing anti-VEGF data and another set with oxaliplatin data. Best-fit curves for each treatment group were calculated and plotted in these figures. As seen in **Figure 1**, 5-FU/CoFactor/anti-VEGF treated mice had the slowest tumor growth curve followed by either 5-FU/CoFactor or 5-FU/anti-VEGF treated mice

We also analyzed the differences between mean tumor volumes following treatment. Comparing the various treatment combinations for the anti-VEGF set of data (**Figure 3**), we observed the mean tumor volume of 5-FU/CoFactor/anti-VEGF treated mice (478.6 ± 102.7 , mean \pm SEM, $n = 7$) was less than 5-FU (752.5 ± 104.2 , $n = 8$), 5-FU/Leucovorin (707.5 ± 93.6 , $n = 8$), 5-FU/CoFactor (522.5 ± 78.2 , $n = 8$), and 5-FU/anti-VEGF (502.5 ± 64.1 , $n=8$) treated mice. Oxaliplatin treated mice had the largest tumors (tumor volume $875.0 + 90.6$, mean + SEM, $n = 8$) (**Figure 4**), indicating that the HT-29 tumor was not responsive to this drug (see Plasencia et al. (2002) American Society for Clinical Oncology Annual Meeting Abstract No. 2188.) This probably accounts for the lack of equivalent tumor inhibition in the treatment group receiving the triple drug combination of 5-FU/CoFactor/Oxaliplatin (735.0 ± 80.3 , $n = 8$) (**Figure 4**), when compared with the triple combination 5-FU/CoFactor/anti-VEGF treated mice, which had the smallest tumor sizes of any anti-VEGF combination (**Figure 3**).

Mouse survival curves were also calculated for all treatment groups. Mice were euthanized upon overt systemic toxicity, tumor ulceration, or when tumor diameter reaches >2 cm. At the completion of the study period (42 days), 75% of mice treated with 5-FU/CoFactor were still alive (**Figure 5**). This survival was significantly longer than mice treated with only 5-FU (25%, $p < 0.05$, Logrank test). In addition to 5-FU/CoFactor treated mice, 5-FU/CoFactor/anti-VEGF treated mice also survived longer (57%) than all other treatment groups. The lack of protection of mice treated with 5-FU/CoFactor/Oxaliplatin (25%) (**Figure 6**) compared to other treatment groups can most likely be attributed to the apparent resistance of the HT-29 tumor to oxaliplatin (**Figure 3**). For the oxaliplatin treatment subgroup analysis, 5-FU/CoFactor treatment provided the greatest survival benefit.

EXAMPLE 2: NUDE MOUSE STUDY ON COLORECTAL TUMOR HT-29 TREATMENT WITH 5-FU, 5,10-CH₂-THFA, FA, ANTI-VEGF, AND OXALIPLATIN.

5 Materials and Methods

Mice

Nude (nu/nu) mice were obtained from Charles River Laboratories. Mice were 6-8 weeks old at the start of all studies. Mice were maintained in isolated, hepa-filter ventilated cages with 4 mice per cage at LAB International's vivarium (San Diego, CA).

10

Cell Lines

The human colon carcinoma HT-29 was obtained from American Tissue Culture Collection (ATCC). Cell lines were maintained in DMEM containing 10% fetal bovine serum (FBS), 2mM l-glutamine, 100 units/ml penicillin, and 100 micrograms/ml streptomycin (DMEM-10) in a 37°C, 5% CO₂ humidified incubator. Cell lines were passaged every 2-3 days prior to *in vivo* experiments.

Drugs

5-Fluorouracil (5-FU) was obtained from Calbiochem. Folinic acid (leucovorin) and oxaliplatin were obtained from Sigma-Aldrich. CoFactor (5, 10 methylenetetrahydrofolate) was manufactured by Eprova AG. A monoclonal antibody to vascular endothelial growth factor (anti-VEGF) was either obtained from R&D Systems (clone 26503 recognizing the human VEGF isoform 165) or Genentech (Avastin).

25

HT-29 Colorectal Carcinoma Nude Mouse Study #2

HT-29 cells were prepared for injection as follows. Confluent tissue culture flasks of HT-29 cells were washed once with PBS followed by cell detachment with trypsin. Detached cells were then washed once in DMEM-10 followed by one wash with PBS. Finally, cells were resuspended at 1x10⁷ cells/ml in PBS. Nude mice (nu/nu) were inoculated subcutaneously with 100microliters (10⁶ cells) of HT-29 cells using a 28 gauge insulin needle/syringe. When tumors reached 30 to 100 mm³ in volume, mice

were treated with various combinations of 5-FU, CoFactor, leucovorin, and anti-VEGF (Genentech's Avastin antibody) administered by intraperitoneal injection. All drugs were dosed daily (0.6 mg/mouse/drug) for seven consecutive days with the exception of anti-VEGF, dosed twice (100 micrograms/mouse) on days 1 and 7. In addition, CoFactor or 5 leucovorin were injected 20 minutes prior to 5-FU injection. Tumor sizes were measured every 2 to 3 days using calipers. Tumor volume was calculated using the following formula: tumor volume = (length x width²)/2. Mice were euthanized by CO₂ followed by cervical dislocation either when a tumor reached >2cm in diameter or upon tumor ulceration.

10

Data Analysis

Statistical analysis of tumor and blood data was performed using GraphPad Prism scientific software. Bonferroni's T test was used to compare tumor sizes between multiple groups. The Logrank test was used to determine statistical differences between 15 group survival curves. In some cases, in which only two groups were compared, Student's T test was used to determine the significance between group measurements.

Results

Based on the pilot results obtained in the first nude mouse study described above, 20 we repeated another HT-29 nude mouse study with some modifications to study design. Modifications included larger group sizes, substitution of Genentech's anti-VEGF Avastin antibody for R&D System's antibody, exclusion of oxaliplatin, increased number of treatment days, and increased the number of doses of the anti-VEGF antibody. Nude mice were treated with the drug combinations described in **Table 3**. In this study, we 25 wanted to examine if combining 5-FU/CoFactor treatment with the anti-VEGF antibody Avastin could inhibit colorectal tumor growth more than other drug combinations. Drug concentrations and treatment days are described in the materials and methods section. Following treatment, tumor sizes were measured every 2-3 days and tumor volumes calculated. Tumor volumes were then plotted versus time from treatment initiation 30 (**Figure 7**). Best-fit curves for each treatment group were calculated and plotted in this figure. Based on the best-fit curve analysis, the average doubling time for each group

was calculated (**Table 4**). Mice treated with the combination of 5-FU/CoFactor/Avastin displayed the slowest growth kinetics (doubling time = 9.9 days) compared to all other groups. These results are consistent with results obtained in the first nude mouse tumor study described earlier.

5 We also analyzed the differences between mean tumor volumes determined 19 days following treatment initiation. The mean tumor volumes \pm SEM are plotted in figure 8. We observed the mean tumor volume of 5-FU/CoFactor/Avastin treated mice (94.0 ± 10.2 , mean \pm SEM, n =12) was significantly less (p<0.05, Bonferonni's T test) than 5-FU (368.5 ± 63.7 , n = 10), 5-FU/Leucovorin (262.0 ± 36.5 , n =11), 5-FU/CoFactor (225.4 ± 32.0 , n=12), 5-FU/Avastin (225.5 ± 28.8 , n=12), but not 5-FU/Leucovorin/Avastin (140.8 ± 20.3 , n=12) treated mice. In contrast, mean tumor volumes of 5-FU/Leucovorin/Avastin treated mice were only significantly smaller than tumor volumes in 5-FU treated mice but not other treatment groups.

15 Mouse survival curves were also calculated for all treatment groups. Mice were euthanized upon overt systemic toxicity, tumor ulceration, or when tumor diameter reached >2cm. Prior to study completion (38 days from treatment initiation), $\leq 50\%$ of mice treated with saline, 5-FU, or 5-FU plus Avastin were still alive (**Figure 9**). In contrast, 92% of mice treated with 5-FU plus Avastin in combination with either CoFactor or leucovorin were still alive. This pattern of survival for the various drug 20 combinations is similar to the results observed in the first nude mouse colorectal tumor study described above.

EXAMPLE 3: BLOOD ANALYSIS OF BALB/C MICE TREATED WITH COMBINATIONS OF 5-FU, LEUCOVORIN, AND COFACTOR

Materials and Methods

Mice

30 Balb/c mice were obtained from Charles River Laboratories. Mice were 6-8 weeks old at the start of all studies. Mice were maintained in isolated, hepa-filter ventilated cages with 4 mice per cage at LAB International's vivarium (San Diego, CA).

Drugs

5-Fluorouracil (5-FU) was obtained from Calbiochem. Folinic acid (leucovorin) and oxaliplatin were obtained from Sigma-Aldrich. CoFactor (5, 10 methylenetetrahydofolate) was manufactured by Eprova AG.

5

Balb/c Blood Analysis Study

Balb/c mice, 7 weeks old female mice, were injected for seven consecutive days with combinations of 5-FU, leucovorin, and CoFactor. All drugs were intraperitoneally injected (100microliters/mouse, 0.6mg/mouse/drug) using a 28 gauge insulin needle/syringe. 200-250microliters blood/mouse was collected by retro-orbital puncture into EDTA-coated microtainer tubes (VWR International) on days 0 (prior to drug injection), 8, and 13. Complete blood counts plus blood differentials were determined by Labcorp Corporation of America using a Bayer Advia 120 Hematology analyzer.

15 Results

In addition to its tumoricidal activity, 5-FU is cytotoxic towards normal cells, especially cells of the hematopoietic system due to its myelosuppressive effects. Because of the related chemical characteristics and modes of action of leucovorin and CoFactor, we wanted to determine if there were similar toxicity profiles of 5-FU/CoFactor combination therapy. As such, we injected normal Balb/c mice with various combinations of 5-FU, leucovorin, and CoFactor (Table 5). Pretreatment, one week, and two weeks following treatment, we analyzed complete blood counts plus differentials for changes in blood parameters. Furthermore, we analyzed qualitative and quantitative measures of drug toxicity.

25 After one week of drug dosing, we observed all mice had drug-related toxicity including ruffled fur, moribundity, and dehydration. Within 12 days of initiation of drug treatment, all mice in the 5-FU only and 5-FU/leucovorin treatment groups had died. In contrast, 38% of mice (5 of 13) in the 5-FU/CoFactor treatment group were alive after 14 days. Kaplan-Meier survival curves were plotted for all treatment groups (Figure 10).
30 Logrank statistical comparison of the 5-FU/CoFactor treatment group versus the 5-FU/Leucovorin treatment group indicated a significant difference in survival ($p < 0.05$).

Blood analysis also revealed differences in select blood cell types (**Figure 11**). We measured the following blood parameters: white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin content (MCHC), neutrophils, lymphocytes, 5 platelets (PLT), eosinophils, basophils, and monocytes. One week following drug treatment, we observed significantly more white blood cells in 5-FU/CoFactor treated mice than 5-FU/leucovorin treated mice ($p < 0.05$, Student's t test). Among the white blood cell subsets, we observed significantly more platelets and neutrophils in the 5-FU/CoFactor treated group than the other treatment groups.

10 Since we observed differences in both platelet and neutrophil levels following 5-FU/CoFactor treatment, we assessed these cell types further. Using NCI grading criteria for toxicity, we calculated the percentage of mice with either combined grade 1/2 toxicity, grade 3 toxicity, or grade 4 toxicity. For platelets, we observed 25% of mice treated 5-FU alone developed grade 4 toxicity (**Figure 12**). In contrast, no grade 4 15 toxicity was noted for either 5-FU/leucovorin or 5-FU/CoFactor treated mice. However, unlike 5-FU/leucovorin mice with grade 3 toxicity (45%), only 15% of 5-FU/CoFactor treated mice developed grade 3 platelet toxicity. The remaining 5-FU/CoFactor treated mice (85%) developing only grade 1 or 2 toxicity. As such, this data suggests 5-FU/CoFactor induces milder platelet toxicity than either 5-FU alone or 5-FU/leucovorin.

20 Similarly, we assessed the neutrophil toxicity profiles. In contrast to the platelet differences, the standard NCI grading system did not reveal noticeable neutrophil differences between treatment groups. For example, 100% of both 5-FU only and 5-FU/leucovorin treated mice developed grade 4 toxicity while 92% of 5-FU/CoFactor treated mice developed the same grade 4 toxicity. The remaining 8% of 5-FU/CoFactor 25 treated mice developed grade 3 toxicity (**Figure 13**). However, closer analysis of mice that developed grade 4 toxicity revealed quantifiable neutrophil differences. We divided mice with grade 4 toxicity into subgroups based on their neutrophil cell count ranges following treatment (**Figure 14**). This analysis revealed that 100% of mice treated with 5-FU only, and 80% of 5-FU/leucovorin treated mice, had neutrophil cell counts between 30 0 and 99. In contrast, only 40% of 5-FU/CoFactor treated mice developed this lowest level neutrophil cell count. The majority of grade 4-rated 5-FU/CoFactor treated mice

(50%) had neutrophil cell counts in the range of 200-499. Thus, this data suggests 5-FU/CoFactor results in milder neutrophil toxicity than either 5-FU alone or 5-FU/leucovorin.

8

Antitumor activity of combination 5,10-methylenetetrahydrofolate, 5-fluorouracil, and anti-vascular endothelial growth factor against human colorectal HT-29 tumors in nude mice.

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5 CA

Background: Folinic acid (leucovorin) has been used as the standard combination therapy as a modulator of 5-fluorouracil (5-FU) for cancer treatment. However, leucovorin is inactive directly and must undergo several metabolic transformations to its

10 active metabolite 5,10-methylenetetrahydrofolate (CoFactor) to be effective. In contrast, CoFactor supplies 5,10-methylenetetrahydrofolate directly and has demonstrated enhancement of the antitumor effects of 5-FU in Phase I/II human clinical trials for colorectal and breast cancer. To determine if the antitumor activity of CoFactor/5-FU could be enhanced further, we examined its use in combination with a recombinant

15 antibody specific for vascular endothelial growth factor (aVEGF), an inhibitor of angiogenesis, against human colorectal HT-29 tumors in nude mice. Methods: 6-8 week old nude mice (nu/nu) were inoculated subcutaneously with 2×10^6 HT-29 cells. When tumors reached 0.1 to 0.3 cm³ in volume, mice were treated with various

20 combinations of 5-FU, CoFactor, leucovorin, and aVEGF administered by intraperitoneal injection. All drugs were dosed daily (0.6 mg/mouse/drug) for five consecutive days with the exception of aVEGF, dosed once (100 mg/mouse) on day 1. In addition, CoFactor or leucovorin were injected 20 minutes prior to 5-FU injection. Tumor volumes were calculated every 2 to 3 days. Results: One month following treatment, we

25 observed smaller mean tumor volumes in mice treated with combination CoFactor/aVEGF/5-FU ($0.48 \text{ cm}^3 \pm 0.1$, n=8, mean \pm SEM) than mice treated with either 5-FU alone ($0.75 \text{ cm}^3 \pm 0.1$), CoFactor/FU ($0.52 \text{ cm}^3 \pm 0.08$), or leucovorin/5-FU ($0.71 \text{ cm}^3 \pm 0.09$). Furthermore, there was greater survival of mice treated with CoFactor/5-FU either with or without aVEGF (57% and 88%, respectively) compared to mice treated with only 5-FU (25%). Conclusions: This study suggests combination

30 CoFactor/aVEGF/5-FU treatment might have utility as a colorectal tumor therapy with greater antitumor activity than standard 5-FU therapies.

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20 All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

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All references cited herein, including those in the bibliography, are incorporated by reference in their entireties.

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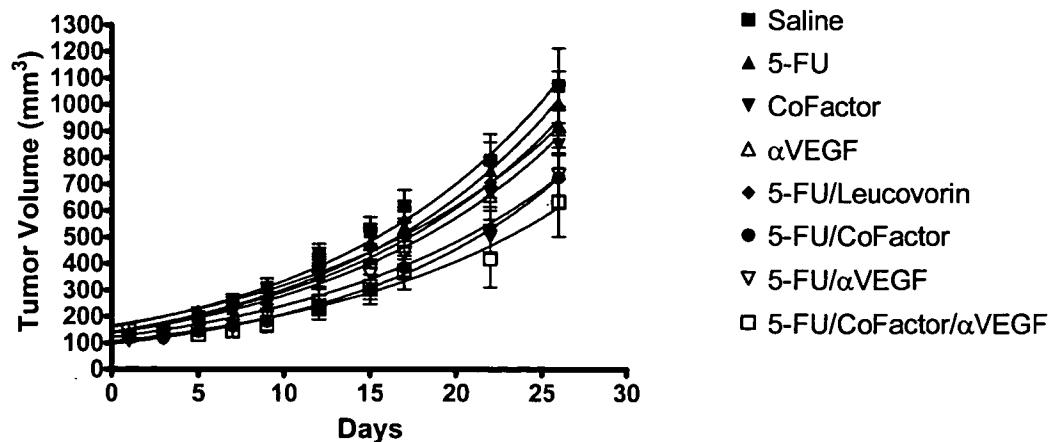


Figure 1. HT-29 Tumor Growth Kinetics. HT-29 tumor volumes were plotted against time from treatment initiation. Mean tumor volume \pm standard error of the mean are plotted. Curves were generated by best-fit analysis.

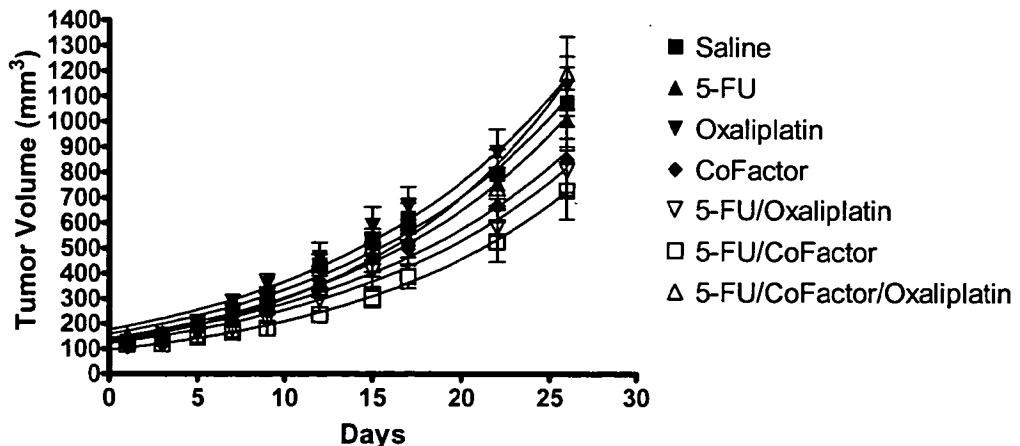


Figure 2. HT-29 Tumor Growth Kinetics. HT-29 tumor volumes were plotted against time from treatment initiation. Mean tumor volume \pm standard error of the mean are plotted. Curves were generated by best-fit analysis.

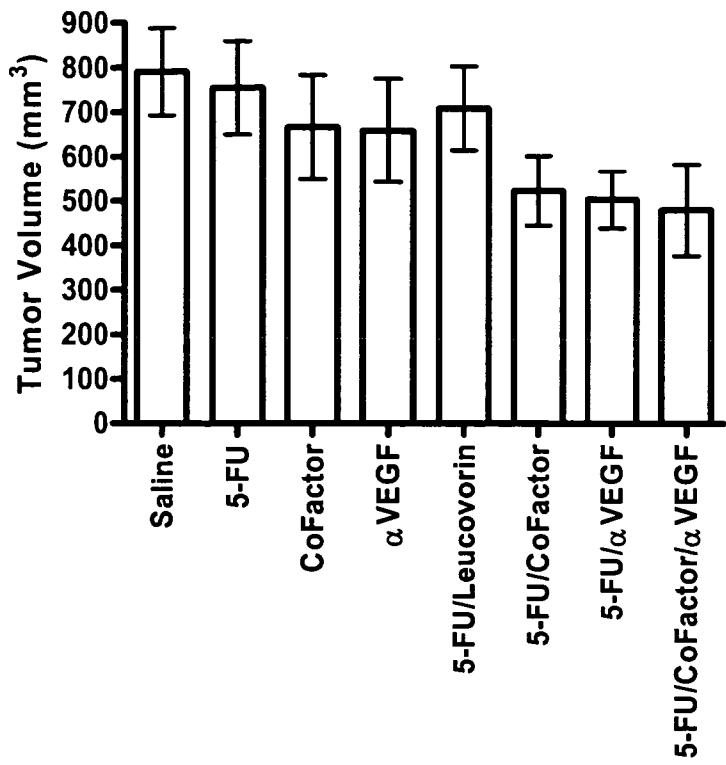


Figure 3. Mean Tumor Volumes Following Treatment. Mean tumor volumes 22 days following treatment initiation were plotted for each treatment group. Error bars represent standard error of the means.

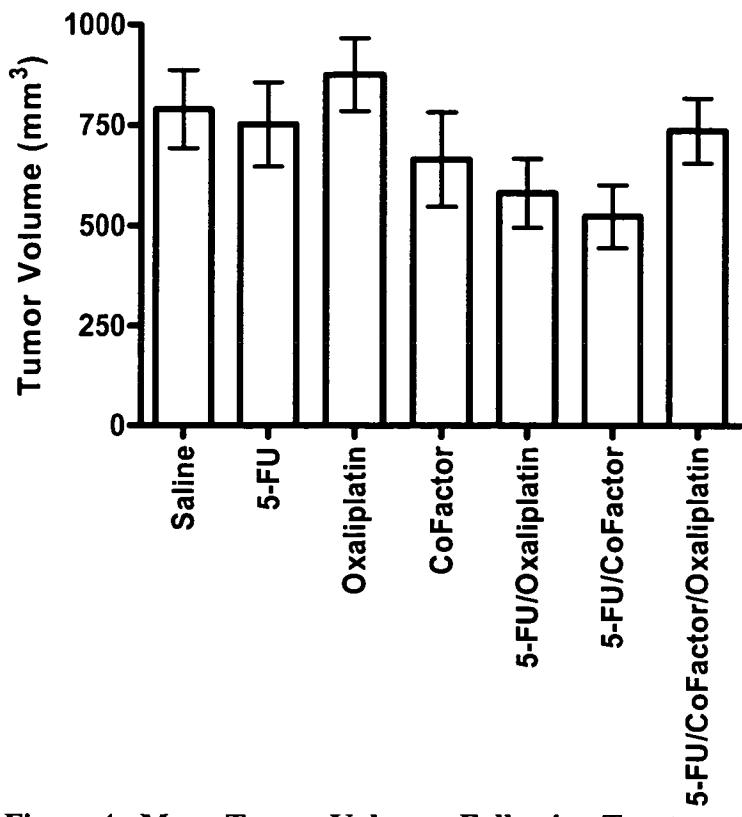


Figure 4. Mean Tumor Volumes Following Treatment. Mean tumor volumes 22 days following treatment initiation were plotted for each treatment group. Error bars represent standard error of the means.

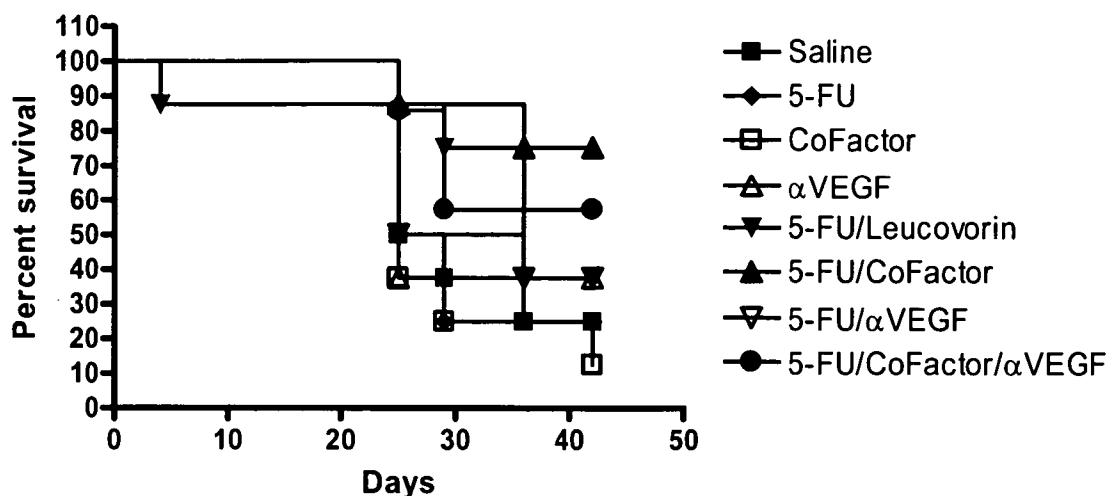


Figure 5. Nude Mice Survival Curves. Kaplan-Meier plot of survival of Nude mice following treatment with combination of 5-FU, CoFactor, leucovorin, and anti-VEGF.

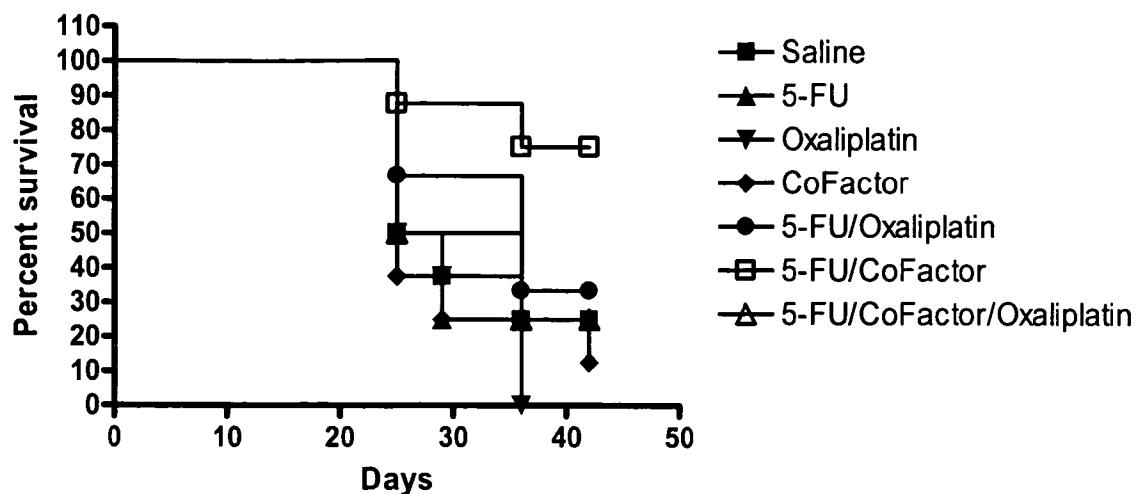


Figure 6. Nude Mice Survival Curves. Kaplan-Meier plot of survival of Nude mice following treatment with combination of 5-FU, CoFactor, and oxaliplatin.

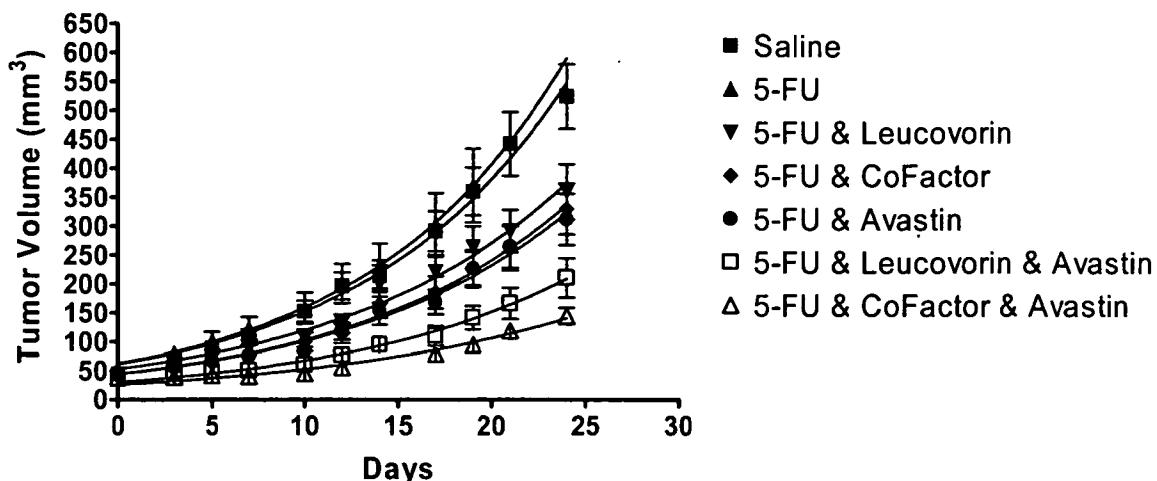


Figure 7. HT-29 Tumor Growth Kinetics. HT-29 tumor volumes were plotted against time from treatment initiation. Mean tumor volume \pm standard error of the mean are plotted. Curves were generated by best-fit analysis.

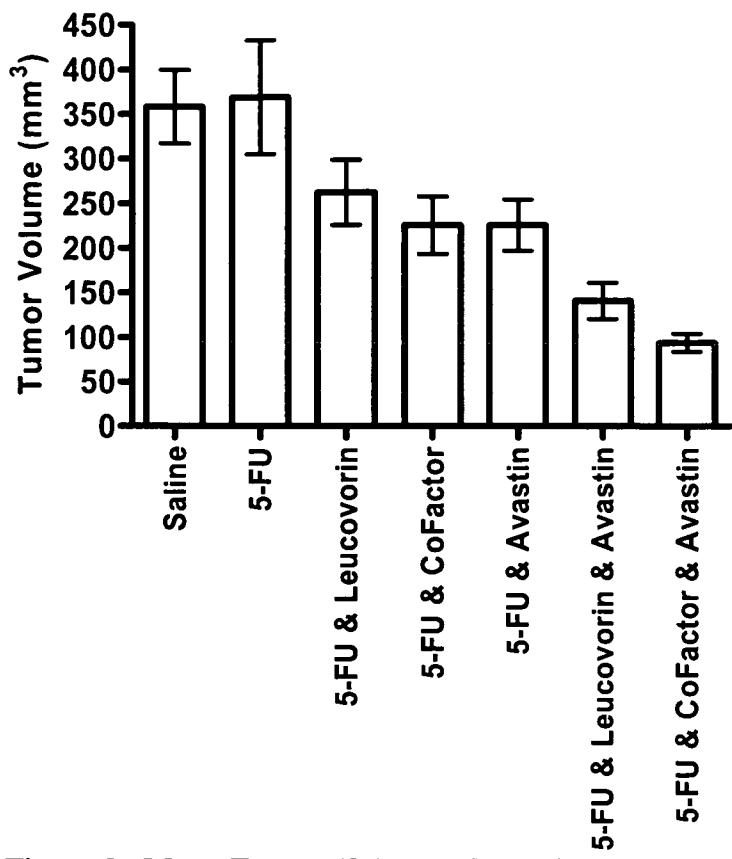


Figure 8. Mean Tumor Volumes Following Treatment. Mean tumor volumes 19 days following treatment initiation were plotted for each treatment group. Error bars represent standard error of the means.

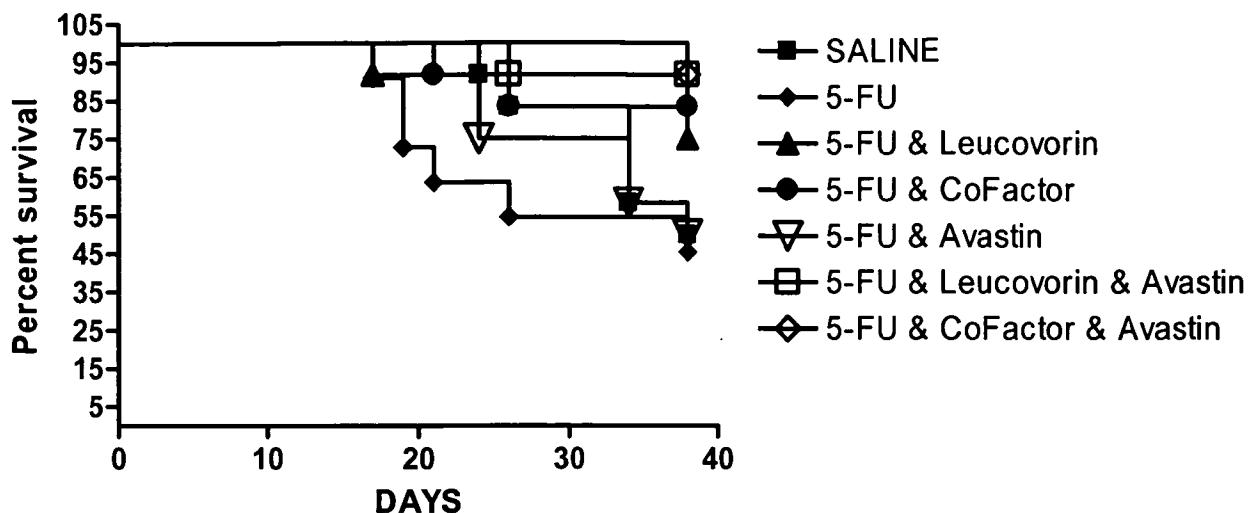


Figure 9. Nude Mice Survival Curves. Kaplan-Meier plot of survival of Nude mice following treatment with combination of 5-FU, CoFactor, and Avastin.

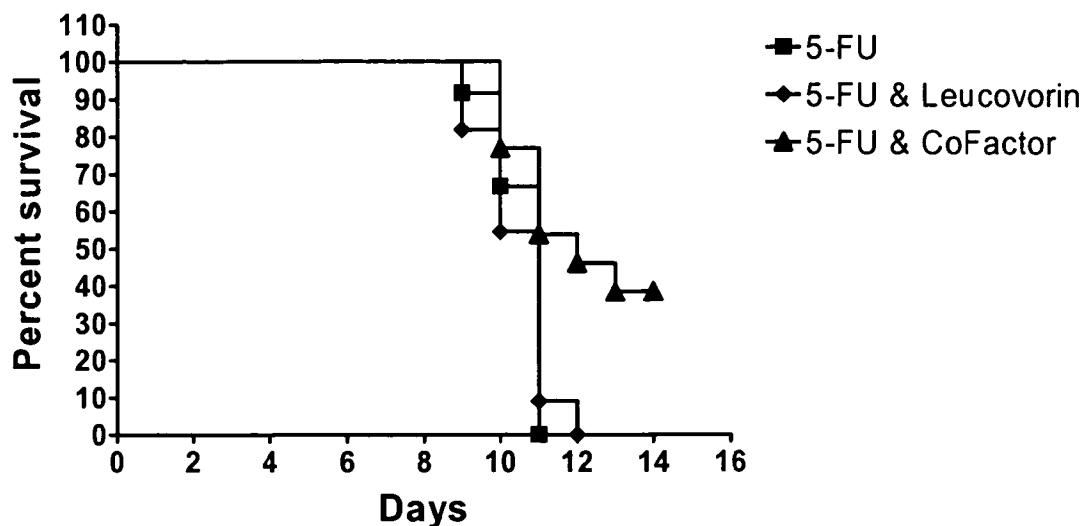


Figure 10. Balb/c Survival Curves. Kaplan-Meier plot of survival of Balb/c mice following 5-FU, 5-FU/leucovorin, and 5-FU/CoFactor treatment.

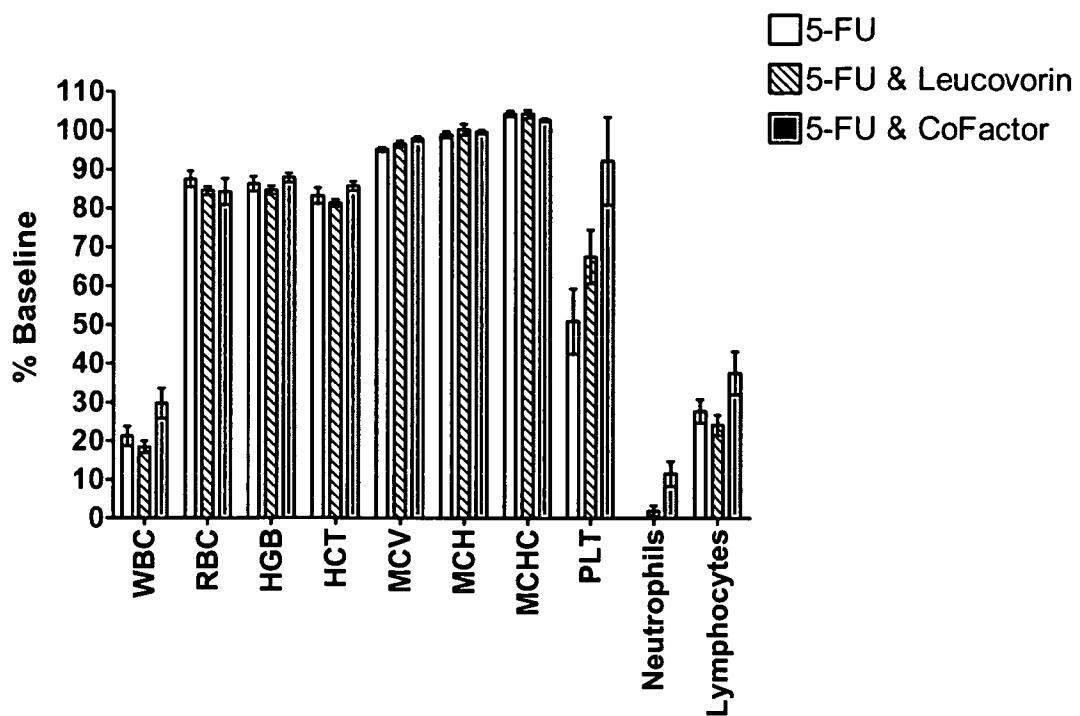


Figure 11. Balb/c Blood Analysis. Blood measurements taken 1 week after drug therapy were divided by the pre-treatment blood measurements to calculate the percentage baseline measurement plotted in the graph. Mean data values \pm standard errors of the means are plotted for each treatment group.

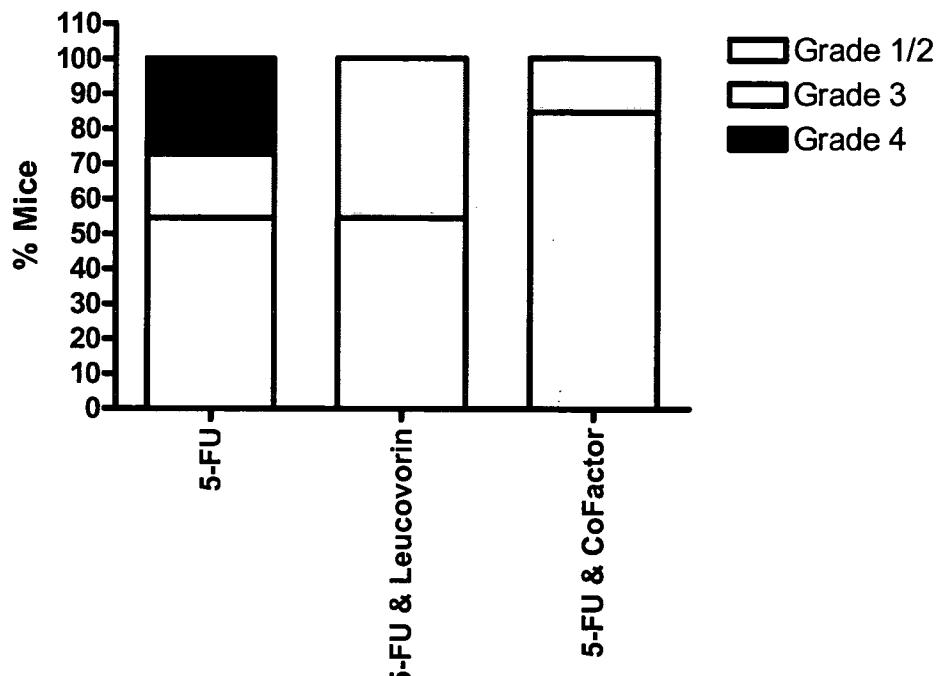


Figure 12. Platelet Toxicity Grading. One week following drug treatment, the grade of platelet toxicity was calculated for each mouse. The percentage of mice with grade 1 or 2, grade 3, and grade 4 toxicity are plotted.

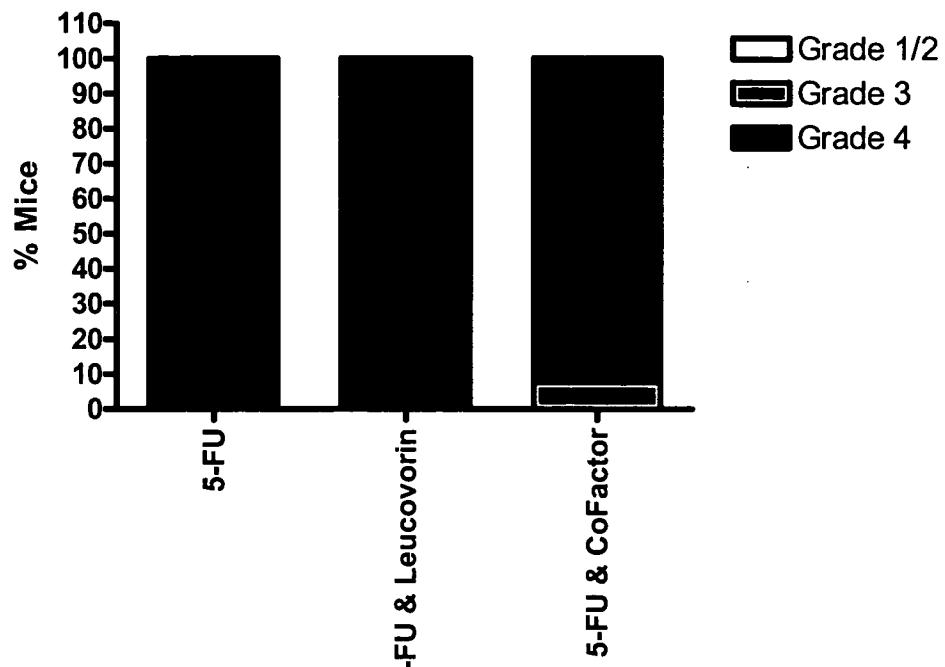
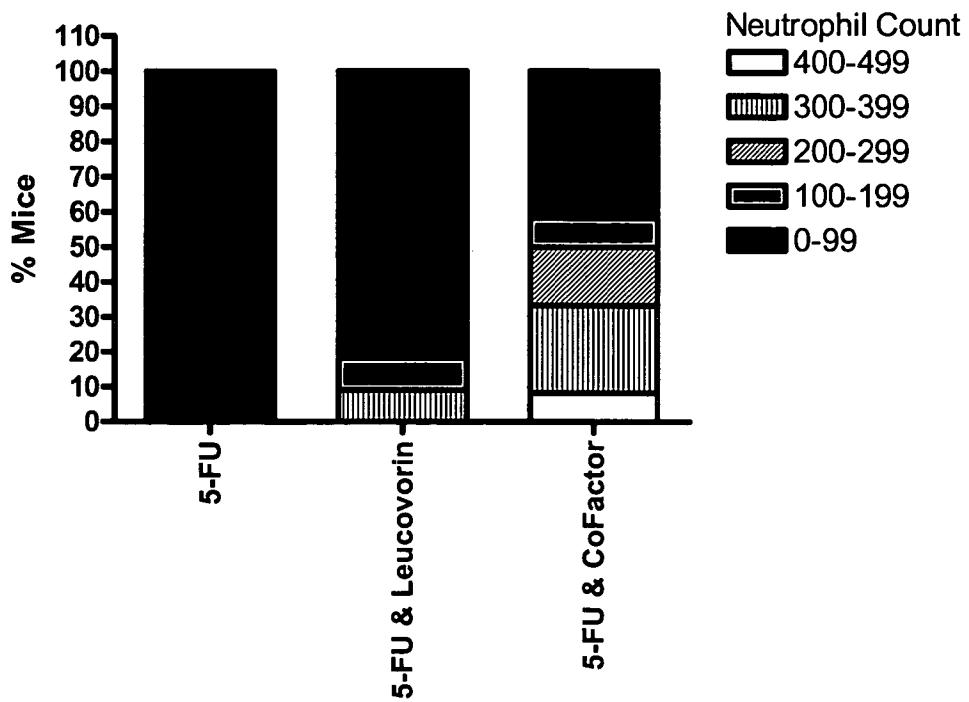


Figure 13. Neutrophil Toxicity Grading. One week following drug treatment, the grade of neutrophil toxicity was calculated for each mouse. The percentage of mice with grade 1 or 2, grade 3, and grade 4 toxicity are plotted.



5 **Figure 14. Grade 4 Neutrophil Toxicity Analysis.** One week following drug treatment, mice with grade 4 neutrophil toxicity were subdivided based on their absolute neutrophil counts. The percentage of these mice with the legend-indicated neutrophil cell counts is plotted.

Table 1. Investigational Colorectal Drugs

Category	Drug	Company	Mechanism
1	ABT-751	Abbott Laboratories	Microtubulin inhibitor
1	Epothilone D	Kosan Biosciences	Microtubulin Inhibitor
2	105AD7	Onyxvax	Anti-idiotype vaccine
2	BCG	Intracel	Mycobacterium Autologous Vaccine
2	EP2101	Epimmune	Peptide Vaccine
2	Mutant ras + IL-2 vaccine	NCI	Dendritic vaccine
2	SGN-00101	Stressgen	BCG vaccine
3	ABX-EGF (panitumumab)	Abgenix	Anti-EGFR
3	GW572016	GlaxoSmithKline	EGFR/ERBB2 inhibitor
3	BAY 43-9006	Bayer/Onyx	RAF/VEGF signal inhibitor
4	EKB-569	Wyeth-Ayerst	EGF Receptor kinase inhibitor
4	Erlotinib	Genentech	Tyrosine kinase inhibitor
4	Gefitinab (Iressa)	AstraZeneca	EGFR tyrosine kinase inhibitor
4	PTK787/ZK 222584	Novartis	VEGFR Tyrosine Kinase Inhibitor
4	E7070	Eisai Medical Research	Cdk2 and cyclin E inhibitor
5	Celecoxib (Celebrex)	Pfizer	Nonsteroidal Anti-inflammatory
5	Rofecoxib (Vioxx)	Merck	Nonsteroidal Anti-inflammatory
6	GM-CSF		Cytokine
6	Interferon alpha		Cytokine
6	Interferon beta		Cytokine
6	TNFerade	Genvec	Adenovirus TNF Cytokine
7	DAVANAT	Pro-Pharmaceuticals	Carbohydrate binder that targets 5-FU to cell
7	Etoposide	Schering Plough	Farnesyl transferase inhibitor
7	LMB-9	NCI	Lewis Y antibody
8	Imatinib (Gleevec)	Novartis	
8	Oblimersin	Genta	BCL-2 inhibitor
9	Tezacitabine	Chiron	Nucleoside Analogue
10	Antineoplaston	Burzynski Research Inst.	
10	Mistletoe extract (Helixor A)	NCCAM	
10	N-phosphonacetyl-L-aspartic acid (PALA)		5-FU modulator
10	PHY906	PhytoCeutica	Anti-diarrhea
10	Talaporfin sodium (LS11)	Light Sciences Corp.	Light activated drug
10	Thalidomide	NCI	Anti-vascular

¹Microtubulin Inhibitor⁶Cytokine²Vaccine⁷Carbohydrate/Lipid³EGFR/VEGFR Target⁸Apoptosis Regulator⁴Tyrosine Kinase/Transcription Factor Inhibitor⁹Nucleoside Analogue⁵Nonsteroidal Anti-Inflammatory¹⁰Miscellaneous

Table 2. Mouse Treatment Groups

<i>Group #</i>	<i>Treatment</i>	<i>Mice/group</i>
1	Saline	8
2	5-FU	8
3	CoFactor	8
4	Anti-VEGF	8
5	Oxaliplatin	8
6	5-FU/Leucovorin	8
7	5-FU/CoFactor	8
8	5-FU/anti-VEGF	8
9	5-FU/Oxaliplatin	8
10	5-FU/CoFactor/anti-VEGF	8
11	5-FU/CoFactor/Oxaliplatin	8
Total		88

Table 3. Mouse Treatment Groups

<i>Group #</i>	<i>Treatment</i>	<i>Mice/group</i>
1	Saline	12
2	5-FU	12
3	5-FU/Leucovorin	12
4	5-FU/CoFactor	12
5	5-FU/Avastin	12
6	5-FU/Leucovorin/Avastin	12
7	5-FU/CoFactor/Avastin	12
Total		84

Table 4. Tumor Doubling Times

<i>Group #</i>	<i>Treatment</i>	<i>Doubling Time (days)</i>
1	Saline	7.6
2	5-FU	7.4
3	5-FU/Leucovorin	8.5
4	5-FU/CoFactor	8.2
5	5-FU/Avastin	8.4
6	5-FU/Leucovorin/Avastin	8.6
7	5-FU/CoFactor/Avastin	9.9

Table 5. Balb/c Mouse Treatment Groups

<i>Group #</i>	<i>Treatment</i>	<i>Mice/group</i>
1	5-FU	12
2	5-FU/Leucovorin	13
3	5-FU/CoFactor	13
Total		38